1083-8791/06/1208-0001$32.00/0
doi:10.1016/j.bbmt.2006.04.005

Letter to the Editor

Allogeneic Hematopoietic Stem Cell Transplantation for Patients with Severe Aplastic Anemia Following Nonmyeloablative Conditioning Using 200-cGy Total Body Irradiation and Fludarabine

In patients with aplastic anemia (AA), allogeneic stem cell transplantation (ASCT) is the treatment of choice for younger patients with HLA-matched siblings. In patients older than 40 years, transplant-related mortality approaches 50% and survival does not differ between patients who receive ASCT and those who receive immunosuppressive therapy [1]. In this age group, immunosuppressive therapy has been advocated as upfront therapy, with ASCT offered only for failed immunosuppressive therapy or relapse [2]. However, this approach gives rise to 2 major concerns: a longer interval from diagnosis to transplantation and an increase in the number of transfusions received. Both factors have been recognized as adverse risk predictors for inferior outcomes in patients undergoing ASCT for AA. The recent introduction of less intense nonmyeloablative (NM) conditioning regimens has not only resulted in lower transplant-related mortality but also widened the age range of potential recipients suitable for transplantation and includes those who are less medically fit [3]. We report our preliminary experience with the use of an NM preparative regimen for ASCT in 8 patients with severe AA.

Between November 2000 and October 2005, 8 patients (median age, 43 years; age range, 25-48 years) with acquired, very severe AA (n = 4) or severe AA (n = 4) were enrolled in a treatment protocol that was approved by the ethics committee of Singapore General Hospital. Written informed consent was obtained in all cases. Patient and treatment characteristics are listed in Table 1.

Unmanipulated filgrastim-mobilized peripheral blood stem cells at a median cell dose of 6.56×10^6/kg $CD34^+$ cells (range, 3.34-22.89 $\times$ 10^6/kg) were infused into recipients after a conditioning regimen that was adapted from McSweeney et al [3], which consisted of fludarabine 30 mg/m^2 (days −4 to −2) and total body irradiation 2 Gy (day 0). Acute graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporine 6.25 mg/kg twice daily from day −3 to day +100, followed by a taper until day +180; mycophenolate mofetil 15 mg/kg twice daily from day 0 to day +50, with a subsequent taper to day 100; and a short course of intravenous methotrexate (MTX; 15 mg/m^2 on day +1, 10 mg/m^2 on days +3, +6, and +11) for all but the first 2 patients (patients 1 and 2), who were given only cyclosporine and mycophenolate mofetil.

The conditioning regimen was well tolerated in all patients, with no grade 3 or 4 regimen-related mucositis, nausea, vomiting, diarrhea, veno-occlusive disease of the liver, hemorrhagic cystitis, or cardiac or pulmonary toxicity seen (National Cancer Institute common toxicity criteria) [4]. All patients achieved prompt and sustained engraftment (Table 2). Median time to a neutrophil count >.5 $\times$ 10^9/L was 18 days (range, 13-21 days), and median time to an unsupported platelet count >20 $\times$ 10^9/L was 13 days (range, 8-25 days). Red blood cell and platelet transfusion independence were achieved in evaluable patients at medians of 8 days (range, 1-17 days) and 10 days (range, 6-20 days), respectively. Chimerism analysis on day +28 showed mixed chimerism (87.5%-95%) in all patients. This mixed chimerism persisted at 3-6 months after transplantation in 6 surviving patients, with 4 of these patients achieving full donor chimerism by 6-12 months. No secondary graft failure was seen.

Grade 2-4 acute GVHD was observed in 3 patients (2 with grade 3 and 1 with grade 2). The 2 patients (patients 1 and 2) who did not receive MTX as GVHD prophylaxis developed grade 3 acute GVHD, leading to subsequent invasive fungal infections and death. Chronic GVHD was observed in 3 patients (2 with limited and 1 with extensive GVHD), including 1 patient who received an allograft from a matched unrelated donor, and all responded to calcineurin inhibitors.

With a median follow-up of 24 months (range, 4-52 months), all 6 patients who received MTX-containing GVHD prophylaxis are alive, are independent of transfusion, and have Karnofsky performance

Table 1. *Baseline Characteristics, Grafts, and GVHD Prophylaxis of 8 Patients with AA**

Patient No.	Diagnosis	Donor	D/R Age (y)	D/R Gender	Time from Diagnosis to HSCT (mo)	Prior Therapy	CD34 Dose Infused ($\times 10^6$/kg)	MNC Dose Infused ($\times 10^8$/kg)	GVHD Prophylaxis	D/R CMV Status
1	SAA	Matched sibling	49/48	M/F	2	No	6.56	16.14	CSP/MMF	+/+
2	SAA	Matched sibling	34/47	M/F	60	ATG/CSP	3.34	34.37	CSP/MMF	+/+
3	SAA	Matched sibling	42/46	F/M	6	ATG/CSP	11.46	8.24	CSP/MMF/MTX	−/−
4	vSAA	Matched sibling	27/35	F/M	3	No	5.27	8.79	CSP/MMF/MTX	−/+
5	vSAA	Matched unrelated	25/43	M/F	6	CSP, GCSF, erythropoietin, oxymetholone	5.9	9.08	CSP/MMF/MTX	−/−
6	vSAA	Matched sibling	55/46	M/F	2	No	6.65	20.25	CSP/MMF/MTX	−/+
7	vSAA	Matched sibling	27/25	F/M	1	No	22.89	6.40	CSP/MMF/MTX	NA
8	PNH with SAA	Matched sibling	24/26	F/F	3	No	7.30	13.33	CSP/MMF/MTX	+/+

*NA indicates data not available; F, female; M, male; D, donor; R, recipient; HSCT, hematopoietic stem cell transplant; SAA, severe aplastic anemia; vSAA, very severe aplastic anemia; PNH, paroxysmal nocturnal hemogloblinuria; MNC, mononuclear cells; GVHD, graft–versus-host disease; CSP, cyclosporine; MMF, mycophenolate mofetil; MTX, methotrexate; CMV, cytomegalovirus; GCSF, granulocyte colony-stimulating factor; ATG, antithymocyte globulin.

Table 2. *Clinical Outcome of 8 Patients with AA Undergoing NM Transplantation**

Patient No.	Days to Neutrophil Count >0.5 × 10^9/L	Days to Platelet Count >20 × 10^9/L	Infective Complications Within 100 Days	aGVHD Grade/Site	cGVHD	Current Status	Cause of Death	Final Chimerism Studies
1	Never†	12	Fungal pneumonia	3/liver	—	Dead, day 70	Invasive fungal infection	100% on day 56
2	Never†	15	Disseminated fungal infections	3/liver	—	Dead, day 64	Invasive fungal infection	100% on day 56
3	16	13	No	1/gut	No	Alive, day 1546	—	100% at 3 y
4	19	19	CMV antigenemia	No	Limited/skin	Alive, day 1343	—	100% at 3 y
5	15	13	No	No	Limited/skin	Alive, day 753	—	100% at 2 y
6	16	12	CMV antigenemia	No	No	Alive, day 657	—	100% at 18 mo
7	21	21	CMV antigenemia	2/gut	Extensive/ oral/skin	Alive, day 516	—	100% at 15 mo
8	21	12	No	No	NE‡	Alive, day 132	—	87.5% at 3 mo

*aGVHD indicates acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; CMV, cytomegalovirus.
†Neutrophil count never decreases to $<.5 \times 10^9$/L.
‡Not evaluable for cGVHD.

scores >90%. Four of the matched sibling recipients and 1 unrelated donor allograft recipient maintained full donor chimerism during the final follow-up. One patient (patient 8) with paroxysmal nocturnal hemoglobinuria had sustained engraftment with mixed chimerism (87.5% donor chimerism) and no detectable paroxysmal nocturnal hemoglobinuria clone by fluorescence-activated cell sorting determination of CD55 and CD59 at 4 months' follow-up.

The feasibility of ASCT using an NM preparative regimen in AA remains to be established, mainly due to concerns of a high risk of graft rejection, in particular among patients who receive multiple transfusions. The results seen in this small cohort of patients have highlighted several important points. (1) This minimal conditioning regimen was sufficiently immunosuppressive in allowing prompt and stable engraftment among patients with AA. (2) It was associated with minimal toxicity, with no patients developing early grade ≥3 regimen-related toxicity, which had been seen in patients after conditioning with cyclophosphamide and antithymocyte globulin [5]. This is potentially beneficial in allowing transplantation to be performed in the outpatient setting. (3) This approach also avoids the use of T-cell–depleting agents such as antithymocyte globulin or alemtuzumab. Omission of these T-cell–depleting agents may have resulted in a more rapid post-transplantation immune reconstitution and reduction of infections such as cytomegalovirus (CMV). Three of 8 patients developed CMV antigenemia with no observed CMV disease. This finding compares favorably with other alemtuzumab- or antithymocyte globulin–based NM transplant series, which reported CMV infection rates that exceeded 50% [6,7]. (4) Addition of MTX into the GVHD prophylaxis regimen could account for the more favorable outcome, with no fatal GVHD seen among the 6 patients who received the MTX-containing GVHD prophylaxis. (5) Five of the 8 patients (63%) in this series were >40 years old at the time of transplantation, and 3 of these patients remained disease free and alive at >20 months' follow-up. These encouraging results provide a rationale for conducting larger studies with this less intensive conditioning regimen as upfront therapy in older patients or patients who have comorbidities precluding high-dose cyclophosphamide conditioning.

The uniform and durable engraftment in the current 8 multiply transfused patients is encouraging. This is partly attributed to the use of peripheral blood stem cells that contained a higher $CD34^+$ cell dose and approximately 10-fold the number of T cells. However, reservations remain with the use of peripheral blood stem cells because of the increased risk of chronic GVHD, although a recent report has quoted a low incidence of 26% chronic GVHD when using similar approach in patients with AA [8]. Clearly, improved GVHD control remains a critical future research objective. Optimizing immunosuppression by prolonging cyclosporine therapy and dose-adapted mycophenolate mofetil might be a promising way to decrease GVHD [9].

In conclusion, our results suggest that ASCT using an NM conditioning regimen results in stable engraftment and favorable outcome in patients with AA, including patients who are >40 years old and those who receive unrelated allografts. These promising results, although based on a small number of patients, merit further investigation and confirmation on a larger cohort of patients with longer follow-up.

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